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Medical Staff Conference

Brucellosis

These discussions are selected from the weekly staff conferences in the Department of Medicine, University of California, San Francisco. Taken from transcriptions, they are prepared by Drs. David W. Martin, Jr., Assistant Professor of Medicine, and H. David Watts, Assistant Professor of Medicine, under the direction of Dr. Lloyd H. Smith, Jr., Professor of Medicine and Chairman of the Department of Medicine. Requests for reprints should be sent to the Department of Medicine, University of California, San Francisco, CA 94143.

DR. SMITH:* *Recently we have had two very interesting cases in the hospital representing brucellosis with osteomyelitis. The first summary will be given by Dr. Michael Jensen.*

DR. JENSEN:† The patient is a 68-year-old man who formerly worked as a renderer in a plant where animal carcasses were processed to tallow for soap. Approximately 28 years before admission he experienced recurrent fevers for which he was seen at Stanford where the diagnosis of brucellosis was made. He was treated with bed rest alone and underwent a spontaneous remission. In October 1972, an enlarged liver and elevated serum transaminase enzymes were noted. A liver biopsy was done and the resulting diagnosis was chronic active hepatitis. In January 1973, a Baker's cyst appeared behind the left knee and upon surgical incision it drained purulent material. Results of cultures were negative. Soon, the onset of recurrent afternoon fevers was noted. In June 1973, while the patient was playing golf, there was a sudden onset of neck pain and he was referred to the University of California. An extensive workup showed a normal leukocyte count with a very high sedimentation rate. Because initially there were thought to be meningeal signs, repeated lumbar punctures were done but findings were normal. The patient was started on steroids for a presumed vasculitis and clinically worsened. Subsequent x-ray films of the cervical spine showed a pronounced destructive process involving the fifth, sixth and seventh cervical vertebrae. One of six blood cultures, as well as the culture of material obtained at open biopsy of the fifth-sixth cervical disc space, showed the presence of Brucella suis. The brucella tube agglutinin (immunoglobulin M) titer was 1:640 and the brucella fluorescent antibody (immunoglobulin G) titer was 1:40. A 21-day course of ampicillin was given, which was followed by clearing of the toxicity and spinal tenderness. Fever cleared after one day of treatment. The patient was discharged with instructions to take ampicillin orally for six weeks. When seen in December 1973, the patient described the recent onset of mild left hip discomfort. Findings on x-ray films of the hip were unremarkable and repeat cervical spine films were unchanged. Orally administered ampicillin, 750 mg four times daily, was restarted and continued during the first five months of 1974. Recurrent afternoon fevers to 38°C (100.4°F) and increasing left hip discomfort were noted, and the patient was readmitted to hospital in May 1974.

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The medical history was remarkable for long-standing diabetes mellitus treated with insulin and diet. On physical examination, the patient appeared youthful and was wearing a cervical collar. A low-grade fever was noted. No lymph nodes were palpable. An atrial gallop was audible and the abdomen was flat. The liver was palpable two fingerbreadths below the right costal margin and the spleen tip was palpable at the left anterior axillary line. Neither was tender. The spine was not tender, but in the left hip there was moderate pain on motion without tenderness to direct palpation.

Neurological examination showed mild left triceps weakness, and the deep tendon reflexes in the upper extremities were mildly hyperactive. Knee jerks were normal, ankle jerks were absent bilaterally and there was a mild peripheral sensory loss. Laboratory data on admission included a hematocrit of 37 percent, a leukocyte count of 5,000 cells per cu mm with 56 percent polymorphonuclear cells, 25 percent lymphocytes, 12 monocytes, 5 eosinophils and 1 band form. Sedimentation rate was 46 mm per hour, serum glutamic oxaloacetic transaminase (SGOT) 102 international units (IU) per liter, alkaline phosphatase 105 IU per liter and hemaglobin antiglobulin was absent. Urinalysis showed 2 to 5 leukocytes per high-power field and 1 plus glucose. X-ray films showed moderate to severe distortion of the fifth, sixth and seventh cervical vertebrae with narrowing of the intervening disc spaces. There was narrowing of the left hip joint space and mild osteoporosis in the acetabulum.

Antibiotics were not administered during the hospital course, and daily afternoon temperature elevations were noted, usually between 37.6 and 38°C (99.7 and 100.4°F). Aspiration of the left hip under fluoroscopy yielded only a few drops of clear fluid without cells, and all cultures of the hip, as well as blood cultures, were negative. Oral administration of tetracycline, 500 mg four times daily, was begun, and there was subjective improvement and partial remission of fever.

DR. SMITH: *Thank you very much. This was a man who had a 25-year history of brucellosis with remission and then later recurrence of brucella osteomyelitis. We have a second patient whose history Dr. Jory Braun will summarize for us.*

DR. BRAUN:* The present illness of this 69-year-old white man, a former farm worker from the San Joaquin Valley, began 27 years before admission when recurrent right knee effusions first began, thought at that time to be the result of trauma. There were no subjective joint symptoms until nine months before admission when low-grade fevers, chills, night sweats, malaise, swelling in his right knee and pain in his right thigh began. In the month before admssion here, he was admitted to Scenic General Hospital in Modesto, where results of purified protein derivative of tuberculin (PPD) skin tests and tests for histoplasmosis, coccidioidomycosis and brucella agglutinins were negative. An x-ray film of the right femur showed intramedullary swelling with periosteal thickening. Cultures taken at incision and drainage of the right thigh were negative on two occasions. The patient was then transferred to the University of California Medical Center.

Upon admission here, the patient appeared chronically but not acutely ill. The temperature was 38°C (100.4°F). Significant physical findings were limited to the right lower extremity. There was a minimal right knee effusion, a fluctuant mass on the medial aspect of the right thigh and granulating wounds from the operative incisions. There was some atrophy of right calf muscles but no decreased range of motion in that leg. There was no peripheral lymphadenopathy and no hepatosplenomegaly. The hematocrit was 38 percent, leukocyte count 11,600 per cu mm with 81 percent polymorphonuclear leukocytes and sedimentation rate 60 mm per hour. Serum calcium and phosphorus, blood urea nitrogen (BUN) and results of urinalysis were within normal limits.

X-ray studies of the right leg showed changes consistent with chronic osteomyelitis of the right femur with a central radiolucency in the medullary cavity and dense calcifications probably due to sequestra. A sinogram showed intramedullary cavitation. On the first day of the patient's stay in hospital, the right thigh lesion was twice aspirated, but cultures from these procedures were negative. On the sixth hospital day, surgical exploration of the right femur was carried out. A sequestrectomy and window procedure were done, the marrow cavity was extensively opened and the operative incision was closed over irrigation tubes. On the 13th day at the University of California Medical Center, brucella agglutinins were found to be positive at 1:1260, and oral administration of tetracycline was begun, 500 mg every six hours. A culture taken during the surgical pro-

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cedure grew Brucella suis ten days later and streptomycin was added to the regimen. Subsequently, the right leg is no longer tender, and there has only been one episode of spontaneous drainage. Several febrile episodes have occurred lasting two to three days, but the patient has generally improved.

DR. SMITH: *Thank you very much. Last week in a Clinical Pathologic Conference here this patient was presented to Dr. Robert Roe from the San Francisco General Hospital, who without benefit of cultures or agglutinations made the correct diagnosis, much to his credit. This morning we are very pleased to have Dr. Stephen Cohen to discuss brucellosis and perhaps put specific emphasis on osteomyelitis.*

DR. COHEN:* This institution has a long and honorable historical association with brucellosis. The disease Malta fever was described over 100 years ago. Some 25 years later Bruce isolated the organism subsequently called Brucella melitensis from the spleen of a fatal case in Malta. Over the next 20 years the other major species of brucella, Brucella abortus and Brucella suis, were recovered from aborted animals. In 1920, the late Dr. Karl F. Meyer and Dr. Edward Shaw, now emeritus Professor of Pediatrics, united these organisms in the genus which they named Brucella. The history of brucellosis is well reviewed in the still classical monograph of Spink.[1]

Epidemiology

Brucella are very small Gram-negative rods which display moderate host specificity. B. melitensis is widely distributed particularly in the Mediterranean littoral in association with goats and sheep. B. abortus is associated with cattle and B. suis with swine. In the mid-1960's epidemic abortion of dogs, particularly of beagles, was traced to Brucella canis, a new species which may be related to B. suis. Other species of brucella are less important.

The last decades have seen pronounced changes in the epidemiology of brucellosis. Although those who work with the animals mentioned are at greatest risk of acquiring brucellosis, brucellosis is a hazard to every man where pasteurization of milk and cheese is not routine. B. abortus is the dominant species causing infection in most of the world, with B. suis being somewhat less prominent. B. melitensis classically causes the most severe disease but is not seen in this country except as it is imported—for example, by returnees from Spain, Italy or Mexico who typically have eaten unpasteurized dairy products. B. abortus was extremely common in this country 30 years ago, but has largely been eradicated through a very vigorous program conducted by the Department of Agriculture in conjunction with Department of Health, Education, and Welfare. The program included serological testing of cattle, slaughter of animals with evidence of infection with brucella, vaccination of range animals and pasteurization of milk. There were 6,500 cases of brucellosis in the United States in 1947 compared with about 200 cases per year at present. By comparison, the United Kingdom with a population one quarter that of ours has five times as many cases as we do. The United Kingdom has not undertaken a vigorous eradication program, and a substantial proportion of its population still drinks unpasteurized milk. In the United States, B. suis is the most common infecting organism, and brucella infection is an occupational disease.

The important epidemiological clue in both patients discussed today, even if the first was without a diagnosis of brucellosis 25 years ago, was the fact that they had worked in proximity to farm animals. As soon as the mention of farm, slaughterhouse or packinghouse is made, the diagnosis of brucellosis should cross the clinician's mind. It is important that he actively pursue the diagnosis because of potential technical difficulties in isolating or identifying the organism. Currently 75 percent of brucella infections in this country result from contact with swine, 75 percent of infections arise in slaughterhouse or packinghouse workers and about 70 percent of isolates are B. suis.[2] Most cases are reported from Iowa, California and Illinois. There has been a notable decrease in the number of cases from Texas—a state which has made a major attempt to eradicate the organism from swine. Swine remain an important source of brucellosis, partly because of the lack of commitment of the industry to its eradication—B. suis is not as serious an economic problem for the farmer as B. abortus is for the cattleman—and partly because serological tests are much less reliable in detecting infected swine. In California approximately 0.2 percent of swine studied were serologically positive. The organisms spread via aerosol and contact. Viable B. suis have been re-

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covered from the tops of fluorescent fixtures 20 feet above the floors of the slaughterhouse.

Brucellae enter the blood stream through mucous membranes or abrasions of the skin, then migrate to the lymphatics and the reticuloendothelial system. The incubation period of brucellosis varies from one to three weeks, but the initial infection is, in many cases, undetected or at least unrecognized. In our second case, we have no clear history of exposure or definite symptoms of an initial infection.

Clinical Presentation

There are four major clinical forms of brucellosis. The first, seen particularly with B. melitensis and B. abortus but also occasionally with B. suis, is an acute bacteremic infection resembling any other, and the blood cultures are readily positive. If the bacteremia is persistent and a high level of antibody develops, a diffuse nephritis may arise, perhaps on an immunologic basis.[3] In addition there may be focal abscesses in the kidney or other tissues. The bacteremic form of the disease is relatively short-lived.

Alternatively, an abacteremic, nonspecific illness may develop, resembling influenza or mononucleosis, or occasionally resembling poliomyelitis because of the profound limb weakness. This is the "serological" form of brucellosis in which the cultures are negative, but the patient has an appropriate epidemiological history and there is serological evidence of active infection.

Third, in about 15 percent of the cases there are focal complications, particularly with B. suis, as in our two patients today. These localizations may occur in any organ but about half of the time involve the skeleton, particularly the vertebral column.[4] Although spondylitis usually responds to medical treatment, bony involvement of other sites is particularly refractory and seems to require aggressive surgical drainage and debridement. Meningitis may arise from the spread of vertebral osteomyelitis. Endocarditis may also occur, and 15 years ago the question was raised whether calcific aortic stenosis might be a specific endocarditic result of brucellosis. Subsequently, it has appeared that such infection is simply coincidental and complicates a preexisting calcified valve. Splenic infection may give rise not only to characteristic calcifications, but to hypersplenism.

Finally, infection may persist—as in our first patient—for many years as a latent infection following an initial bout of brucellosis. Possibly a small abscess in the skeletal system or an intracellular focus in the reticuloendothelial system is reactivated.

In addition to its varied clinical manifestations, brucella organisms are unusual in that they are one of the few pathogens for which a specific biochemical determinant of disease has been found.[5] Brucella infection of goats, cattle and sows leads to placentitis and abortion. This localization seems to correlate with the normal presence of erythritol in the placentas but not in other tissues of these animals. Erythritol is a four-carbon sugar which brucella metabolize in preference to glucose. The correlation with erythritol is not complete, because the canine placenta does not contain erythritol but can develop a brucella placentitis.

Diagnostic Procedures

The diagnosis may come from a biopsy specimen of the involved tissues showing granulomata with or without caseation (Figure 1). Caseation is unusual, but when found, is usually associated with B. suis. X-ray studies may show a classical wagon-wheel calcium deposit in the spleen, but most patients do not have this finding.

The brucellergen skin test, used for a long time

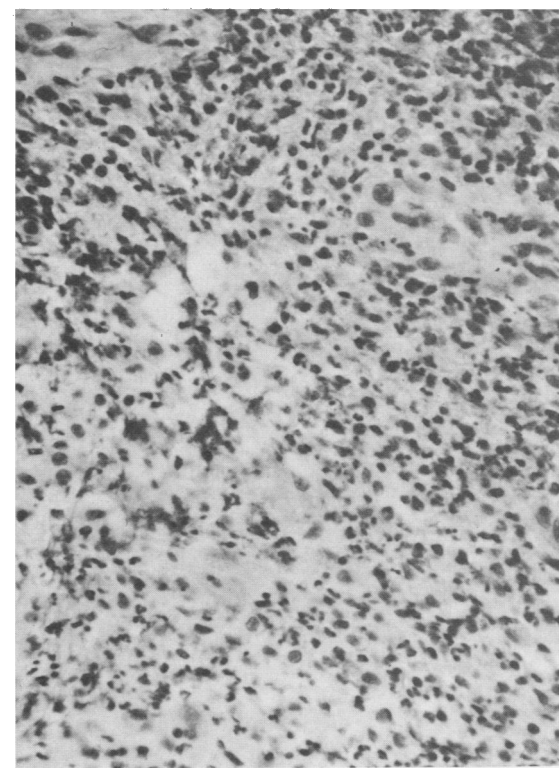

Figure 1.—Granulomatous inflammatory response in biopsy specimen from fifth cervical vertebra, Case 1.

TABLE 1.—*Serodiagnosis of Brucellosis*

Status of Infection	*Agglutination (IgM)*	*Complement-Fixation (IgG)*
Prior infection, not active	+	−
Following skin test or repeated exposure in patient with inactive infection	±	±
Active disease, acute or chonic .	±	+

in the diagnosis of brucellosis, is unreliable and, furthermore, like the tuberculin test, usually remains positive following the initial infection. Uncritical use of the skin test has resulted in an erroneous diagnosis of active brucellosis in many previously infected but recovered persons with neurasthenic syndromes. Like the histoplasmin skin test, the brucella skin test will induce a rise in serological titer, and may further mislead one if serum is not drawn first. For these reasons the skin test has fallen into disfavor[6] and the antigen is no longer available in the United States.

The most useful diagnostic tests for brucellosis are serologic,[7] but it is important to keep in mind that Gram-negative organisms such as brucella give a different serological progression of response from that which we normally expect. In most infections, initially formed immunoglobulin M (IgM) antibodies are replaced in turn by immunoglobulin G (IgG) antibodies, which persist for life. Usually, then, the presence of IgM antibody reflects active or recent infection, whereas the presence of IgG alone suggests remote infection. In contrast, following an infection with Escherichia coli, Salmonella typhosa or brucella, the usual early IgM antibody formation continues throughout life, and IgG antibodies do not usually persist beyond the hyperimmunizing stimulus of acute disease. Thus, in *active* brucellosis, 7S (IgG and immunoglobulin A [IgA]) antibodies are produced.[8] The IgG antibodies fix complement better than the IgM and may be detected by the complement fixation test, the indirect fluorescent antibody test or an immunodiffusion study. Blocking antibodies—probably primarily IgA, although this has not been conclusively shown—are also produced during active infection. These nonagglutinating IgA antibodies bind to the surface of the bacteria utilized in the agglutination test and thus prevent IgM antibodies from reacting and forming the cross lattices responsible for agglutination. Therefore, the serum of a patient previously shown to agglutinate brucella may show a prozone particularly at lower titers and actually give a negative reaction following reactivation of the latent infection. If a laboratory were to screen for brucella antibodies at a single low titer, it consequently might obtain a false-negative serologic result. Slide agglutination tests are particularly prone to give false-negative results. Furthermore, all patients do not react with all antigens, even those derived from the same species of pathogen.

To summarize the serodiagnosis of brucellosis (Table 1), prior infection without current active disease is usually attended by the presence of IgM antibody without IgG. If the patient with prior exposure is given a skin test, not only will IgM antibodies be found which may be boosted to a high titer, but the skin test may induce IgG antibodies. If the patient has active disease, IgM antibodies may or may not be shown, due to the prozone phenomenon, but there will be complement-fixing and precipitating IgG antibodies. The same serologic features occur with chronic focal disease, as in our patients. Unfortunately, diagnostic confusion is common in high-risk persons such as veterinarians and farmers who have had remote brucellosis. They are not actively infected but are continually re-exposed and restimulated to develop serological changes resembling those seen in active cases. In such patients, one can only make a conclusive diagnosis by culture.

Brucellae usually require five to seven days to give a positive blood culture. Some strains of B. abortus require carbon dioxide for growth, and if one is considering the diagnosis of brucellosis, it is worthwhile to alert the laboratory to ensure optimal handling of the specimen. The frequently stated difficulty in obtaining positive cultures is not only technical, however. Brucella organisms are not extraordinarily fastidious, and their reputation is largely due to the fact that even in patients with prolonged and recurrent fever bacteria are rarely present in the blood stream, and even then are usually present in very small numbers. Therefore the habit of holding negative blood cultures for long periods of time is unnecessary.

Our first patient demonstrates the serodiagnostic confusion that can arise. The slide agglutination was positive at 1:320 in our laboratory but negative at 1:160; if screening had been done only at some lower titer, the test would be called negative. Tube agglutination results gave somewhat higher titers without prozone formation. However, agglutination tests at the laboratories of the Hooper Foundation and the Department of Microbiology at the University of California,

Berkeley, were negative on this patient's sera. Presumably the antigen used by these laboratories simply did not react at any titer. Complement-fixing antibodies were present in tests at both of these laboratories as expected for active infection.

Immunity to brucellosis is less than completely understood, and is well-reviewed by Elberg,[9] a former pupil of Dr. Meyer. We know that the antibodies which are produced against brucella do facilitate phagocytosis of the organism by macrophages, but the antibodies do not seem to be highly protective. Cellular immunity, mediated by the interaction of immune lymphocytes and macrophages, appears to be the most important determinant of the disposition of this infection, as it is for a host of other infections capable of latency and chronicity, such as tuberculosis and fungal diseases.

Therapy

If patients who become acutely ill are not treated at all, their undulant fever will usually subside within several weeks to a few months, and most patients will not become ill again. Antibiotics provide a prompt defervescence, but unfortunately it is not clear whether the proportion of patients developing chronic disease, probably some 15 percent, is altered significantly.

The usual antimicrobial therapy of brucellosis in adults is 2 grams of tetracycline in divided doses, administered orally for three to six weeks. Because of the tendency to relapse, many physicians select a longer rather than shorter period of treatment. It was once firmly believed that streptomycin should be given in addition to tetracycline, particularly in severe cases. However, the virtues of adding streptomycin have become less clear over the years, and no definitive studies show that the ultimate results are superior to those obtained with tetracycline alone. The inhibitory and bactericidal concentrations of tetracycline for brucella isolates are generally quite low, below 2 micrograms per ml.

In the first case presented today, we administered somewhat radical therapy by initially employing ampicillin. Although ampicillin has been previously reported to be ineffective in clinical brucellosis, one negative study appears to have been in patients without active disease who could not be expected to benefit,[10] while the other includes no data on antibiotic susceptibility of the organisms.[11] We used ampicillin because the literature[12] stated and our *in vitro* laboratory tests showed that the organism was highly sensitive to ampicillin and because the organism was relatively easily killed by ampicillin compared with poorer prospects for killing it by safely attainable levels of tetracycline. The patient had an extremely prompt and gratifying clinical response.

We hoped that bactericidal therapy might make his return for treatment less likely, but pain subsequently developed in the patient's hip. In view of his age and despite the absence of a definable lesion, we reinstituted ampicillin without waiting for a positive cultural diagnosis. Unfortunately, after again improving promptly and receiving six months of additional ampicillin, the patient returned with probable infectious arthritis and osteomyelitis in the head of the femur. Our assumption, although we could not culture the organism out of our patient, was that since new disease developed while the patient was on ampicillin, it might well be a resistant mutant. We therefore chose to change the antibiotic regimen to tetracycline. He has notably improved, and we hope that he will continue to do so. Nonetheless, chronic focal bony disease requires surgical excision if it is to be cured at all. It is unlikely that any course of any antibiotic will prevent this patient from relapsing, particularly if antibiotics are stopped. Our ability to terminate this infectious process depends upon our developing a much greater understanding of the persistence of latent brucella infection and not upon recurrent courses of antibiotics.

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